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(54) Title: PURINE INHIBITORS OF CYCLIN DEPENDENT KINASE 2 AND IκB-α

(57) Abstract

A 2,6,9-trisubstituted purine composition that is useful for inhibiting cell proliferative disorders and as an antifungal agent.

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PURINE INHIBITORS OF CYCLIN DEPENDENT KINASE 2 and IκB-α

BACKGROUND OF THE INVENTION

This is a continuation-in-part of co-pending U.S. Patent Application Serial No. 08/692,012 filed August 2, 1996.

(1) Field of the Invention

This invention concerns 2,6,9-trisubstituted purines that have been discovered to be selective inhibitors of cell cycle kinases and, as such, the compounds are inhibitors of cell proliferation. The 2,6,9-trisubstituted purines are useful in for example in - treating autoimmune diseases, e.g. rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, etc., in treating cancer, cardiovascular disease, such as restenosis, host vs graft disease, gout, polycystic kidney disease and other proliferative diseases whose pathogenesis involves abnormal cell proliferation.

This invention also concerns 2,6,9-trisubstituted purines that have been discovered to be potent and specific inhibitors of IκB-α kinase which prevents signal induced NF-κB activation and cytokine synthesis in vitro and in vivo. Such inhibitors are expected to inhibit the synthesis of cytokines and adhesion proteins whose synthesis is transcriptionally regulated by NF-κB. Proinflammatory cytokines such as IL-1, IL-6, TNF and adhesion proteins (e.g. ICAM, VCAM and selections) belong to this class of molecules and have been implicated in the pathogenesis of inflammatory diseases. Thus a potent inhibitor of IκB-α kinase is useful in the clinical management of diseases where NF-κB activation is required for disease

induction.

(2) **Description of the Art**

In the past few years, advances in molecular and cellular biology have contributed to our understanding of the mechanisms of cell proliferation and of specific events that occur during progression of cells through mitosis. *E.g.*, "Progress in Cell Cycle Research" Vol 1, Eds. L. Meijer, S. Guidet and H.Y.L. Tung; Plenum Press, New York, 1995. These studies have shown that progression through the cell cycle is controlled by a family of serine/threonine kinases called cyclin dependent kinases. These enzymes contain (a) a catalytic protein called cyclin dependent kinase (CDK) that uses ATP as a substrate and (b) a regulatory protein called cyclin. Different cyclin-CDK combinations control events such as growth, DNA replication and cell division. One key member of the CDK family of enzymes is CDK2. CDK2 activity has been shown to be essential for mammalian cell cycle progression at the G1/S boundary. Microinjection of antibodies directed against CDK2 blocks the progression of human diploid fibroblasts into the S phase of the cell cycle. Expression of a CDK2 dominant negative mutant in human osteosarcoma cells has a similar effect. Together, these studies indicate that inhibition of cellular CDK2 activity will prevent progression of cells through the mitotic cycle and induce growth arrest prior to the S phase. Consistent with this view, in vitro studies with olomoucine (2-(hydroxyethylamino)-6-benzylamino-9-methylpurine), have shown that it is a specific inhibitor of CDK2 with an IC_{50} of approximately 2.1 µg/ml J. Vesely, et al.; Eur. J.Biochem 224, 771-786 (1994), L. Meijer "Chemical Inhibitors of Cyclin-Dependent Kinases" pp 351-356 in "Progress in Cell Cycle Research Vol 1, Eds. L. Meijer, S. Guidet and H.Y.L. Tung; Plenum Press, New York, 1995. In vivo studies using mammalian cells in culture have shown that olomoucine inhibits cell

proliferation at an approximate concentration of 50 μg/ml.

In this invention, we have developed several compounds whose biological activity is considerably more potent than olomoucine. In vivo studies using mammalian cells indicate that some of the disclosed compounds inhibit cell proliferation at concentrations that are significantly lower than olomoucine.

Recently an IκB-α kinase activity has been described in the cytoplasm of stimulated human umbilical vein endothelial cells (Bennett et al (1996) J. Biol.Chem 271, 19680-19688). Some of the compounds of this invention have been identified as potent and specific inhibitors of IκB-α kinase which prevents signal induced NF-κB activation and cytokine synthesis in vitro and in vivo. The activation of the heterodimeric transcription factor NF-κB is a complex process. In unstimulated cells, the NF-κB (p50/p65) heterodimer is located in the cytosol where it is complexed with an inhibitory subunit IκB-α, IκB-α, binds to NF-κB thus masking its nuclear localization signal and preventing translocation to the nucleus. Upon stimulation of cells with a variety of signals (e.g. lipopolysaccharide) IκB-α is rapidly phosphorylated, uniquitinated and degraded by the proteasome. Degradation of IκB-α, allows the translocation of NF-κB to the nucleus where it activates transcription of a number of inflammatory response genes.

These observations suggest that IκB-α kinase is an attractive target for the identification of inhibitors that may be useful in the treatment of inflammatory diseases where NF-κB activation is required for disease induction.

SUMMARY OF THE INVENTION

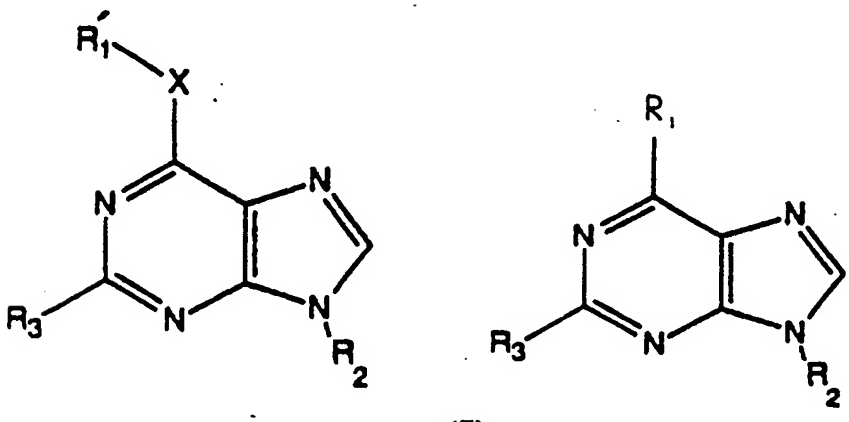
It is an object of this invention to provide 2,6,9-trisubstituted purine compounds, which inhibit the cyclin dependent kinase 2.

It is another object of this invention to provide 2,6,9-trisubstituted purine compounds which are useful for inhibiting cell proliferation.

This invention also constitutes a pharmaceutical composition, which comprises a 2,6,9-trisubstituted purine compound and a pharmaceutically acceptable carrier.

This invention further constitutes a method for inhibiting cell proliferation, which comprises administering to a mammal in need thereof an effective amount of a 2,6,9-trisubstituted purine compound.

In one embodiment, this invention is A 2,6,9-trisubstituted purine composition of matter having the following formula:



(I)

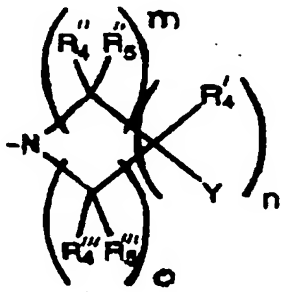
where X is a amino, oxo, thio, or sulfone moiety;

R_1 is halogen or R_1'-X wherein X is an amino, oxo, thio, or sulfone moiety. X is preferably amino.

R_1' is a lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, aryl, substituted aryl, heterocycle, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, heteroalkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, or alkyl cycloheteroalkyl, each having from 1 to 20 carbon atoms;

R_2 is hydrogen, lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocycle, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, heteroalkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, or alkyl cycloheteroalkyl;

R_3 is halogen, hydroxyl, thio, alkoxy, alkylthio, lower alkyl, $-NR_4R_5$ or a component having the formula:



where m=1-3, n=1-3, and o=1-3; Y=carbonyl, $-NR_4R_5$, hydroxyl, thiol, alkoxy, alkythio, and wherein R_4 and R_5 are each (independently) selected from the group including hydrogen, lower alkyl, substituted lower alkyl, alkoxy, amino, amido, carboxyl, cycloalkyl,

substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, or cyano; having from 1 to 20 carbon atoms, and preferably form 2 to 6 carbon atoms. Furthermore, when Y is carbonyl, R'$_4$ does not exist in the composition. R_4'' and R_5'' may be a single oxygen atom and R_4''' and R_5''' may be a single oxygen atom. R_4 and R_5 are preferably the same or different substituted lower alkyl having 2 to 6 carbon atoms and preferably CH_2, CH_2OH, $CH_2HC(CH_3)OH$ and mixtures thereof. There are some limitations to the scope of R_1, R_1', R_2, R_3 when R_3 is 2-hydroxyethylamino and R_2 is methyl, R_1'-X cannot be amino, 3-methyl-2-butenylamino, benzylamino, or 3-hydroxybenzylamino. When R_3 is 2-hydroxyethylamino and R_2 is isopropyl, R_1'-X is not benzylamino, 3-hydroxybenzylamino, or 3-methylbutylamino. When R_3 is 2-hydroxyethylamino and R_2 is 2-hydroxyethyl, R_1'-X cannot be benzylamino. When R_3 is selected from the group consisting of 2-propanol-2-methylamino and 2-dimethylaminoethylamino and R_2 is methyl, then R_1'-X cannot be benzylamino.

In another embodiment, this invention is a method for inhibiting cell proliferation in mammals comprising administering a therapeutically effective amount of the composition of claim 1 to the mammal. The method is useful for treating cell proliferation disorders such at rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, cancer, restenosis, host graft disease, and gout.

In yet another embodiment, this invention is a pharmaceutical composition of matter comprising the composition above in an admixture with one or more pharmaceutical excipients.

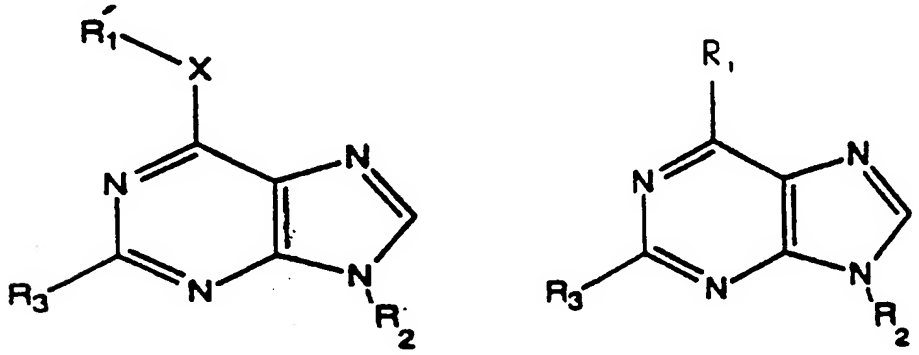
In still another embodiment, this invention is a composition useful for treating fungal infections (fungi) in humans, animals, and in plants.

DESCRIPTION OF THE FIGURE

Figure 1 is a plot of the mean neointimal area of a rat carotid artery treated with a saline vehicle and treated with compound 3 prepared according to Example 2 wherein the unshaded bar represents the untreated section of the carotid artery and the shaded bar represents the treated section of the carotid artery.

DESCRIPTION OF THE CURRENT EMBODIMENT

The present invention relates to a 2,6,9-trisubstituted purine compound having the following formulas:



(I)

where:

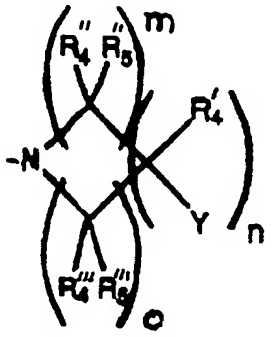
R_1 is halogen or R_1' -X wherein X is a amino, oxo, thio, or sulfone moiety. X is preferably amino.

R_1' may be a lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, aryl, substituted aryl, heterocycle, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, heteroalkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, or alkyl cycloheteroalkyl, each having from 1 to 20 carbon atoms. R_1' is preferably CH_2-aryl, CH_2- substituted aryl, 4-methoxybenzyl, 4-chlorobenzyl, 4-nitro benzyl, 4-(2-pyridinyl) benzyl, aryl, substituted aryl, 3-thiomethoxyphenyl, or 4-thiomethoxyphenyl.

R_2 may be hydrogen, lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocycle, hetaryl, substituted hetaryl, aralkyl,

heteroaralkyl, substituted heteroaralkyl, heteroalkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, or alkyl cycloheteroalkyl where the hydrocarbon compounds have from 1 to 20 carbon atoms. R_2 is preferably isopropyl.

R_3 is halogen, hydroxyl, thio, alkoxy, alkylthio, lower alkyl, $-NR_4R_5$ or a component having the formula:



where m=1-3, n=1-3, o=1-3, Y=carbonyl, $-NR_4R_5$, hydroxyl, thiol, alkoxy, alkythio, and wherein R_4 and R_5 are each selected from the group including hydrogen, lower alkyl, substituted lower alkyl, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, or cyano having from 1 to 20 carbon atoms, and preferably from 2 to 6 carbon atoms. Furthermore, when Y is carbonyl, R'_4 does not exist in the composition. R_4'' and R_5'' may be a single oxygen atom and R_4''' and R_5''' may be a single oxygen atom. R_4 and R_5 are preferably the same or different substituted lower alkyl having from 2 to 6 carbon atoms including $-CH_2CH_2OH$ and $-CH_2CH(CH_3)OH$.

There are some limitations to the scope of R_1, R_1', R_2 and R_3. When R_3 is

2-hydroxyethylamino and R_2 is methyl, R_1'-X cannot be amino, 3-methyl-2-butenylamino, benzylamino, or m-hydroxybenzyl-amino. When R_3 is 2-hydroxyethylamino and R_2 is isopropyl, R_1'-X cannot be benzylamino, m-hydroxybenzylamino, or 3-methylbutylamino. When R_3 is 2-hydroxyethylamino and R_2 is 2-hydroxyethyl, R_1'-X cannot be benzylamino. When R_3 is 2-propanol-2-methylamino or 2-dimethylaminoethylamino and R_2 is methyl, R_1'-X cannot be benzylamino.

The following are definitions for certain terms used herein.

"Halogen" refers to fluorine, bromine, chlorine, and iodine atoms.

"Hydroxyl" refers to the group -OH.

"Thiol" or "mercapto" refers to the group -SH.

"Lower alkyl" refers to a cyclic, branched or straight chain, alkyl group of one to ten carbon atoms. This term is further exemplified by such groups as methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), cyclopropylmethyl, i-amyl, n-amyl, hexyl and the like.

"Substituted lower alkyl" refers to lower alkyl as just described including one or more groups such as hydroxyl, thiol, alkylthiol, halogen, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, carboxyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, cyano. These groups may be attached to any carbon atom of the lower alkyl moiety.

"Alkyl alkenyl" refers to a group -R-CR'=CR''' R'''', where R is lower alkyl, or substituted lower alkyl, R', R''', R''''may independently be hydrogen, halogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined

below.

"Alkyl alkynyl" refers to a groups -RC≡CR' where R is lower alkyl or substituted lower alkyl, R' is hydrogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

"Alkoxy" denotes the group -OR, where R is lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroalkyl, heteroarylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, or substituted cycloheteroalkyl as defined.

"Alkylthio" denotes the group -SR, $-S(O)_{n=1-2}-R$, where R is lower alkyl, substituted lower alkyl, aryl, substituted aryl, aralkyl or substituted aralkyl as defined herein.

"Acyl" denotes groups -C(O)R, where R is hydrogen, lower alkyl substituted lower alkyl, aryl, substituted aryl and the like as defined herein.

"Aryloxy" denotes groups -OAr, where Ar is an aryl, substituted aryl, heteroaryl, or substituted heteroaryl group as defined herein.

"Amino" denotes the group NRR', where R and R' may independently by hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined herein or acyl.

"Amido" denotes the group -C(O)NRR', where R and R' may independently by hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl as defined herein.

"Carboxyl" denotes the group -C(O)OR, where R is hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, and substituted hetaryl as defined herein.

"Aryl" or "Ar" refers to an aromatic carbocyclic group having at least one aromatic ring (*e.g.*, phenyl or biphenyl) or multiple condensed rings in which at least one ring is

aromatic, (*e.g.*, 1,2,3,4-tetrahydronaphthyl, naphthyl, anthryl, or phenanthryl).

"Substituted aryl" refers to aryl optionally substituted with one or more functional groups, *e.g.*, halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heterocycle" refers to a saturated, unsaturated, or aromatic carbocyclic group having a single ring (*e.g.*, morpholino, pyridyl or furyl) or multiple condensed rings (*e.g.*, naphthpyridyl, quinoxalyl, quinolinyl, indolizinyl or benzo[b]thienyl) and having at least one hetero atom, such as N, O or S, within the ring, which can optionally be unsubstituted or substituted with, *e.g.*, halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heteroaryl" or "hetar" refers to a heterocycle in which at least one heterocyclic ring is aromatic.

"Substituted heteroaryl" refers to a heterocycle optionally mono or poly substituted with one or more functional groups, *e.g.*, halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Aralkyl" refers to the group -R-Ar where Ar is an aryl group and R is lower alkyl or substituted lower alkyl group. Aryl groups can optionally be unsubstituted or substituted with, *e.g.*, halogen, lower alkyl, alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heteroalkyl" refers to the group -R-Het where Het is a heterocycle group and R is a lower alkyl group. Heteroalkyl groups can optionally be unsubstituted or substituted with *e.g.*, halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heteroarylalkyl" refers to the group -R-HetAr where HetAr is an heteroaryl group and R lower alkyl or substituted lower alkyl. Heteroarylalkyl groups can optionally be unsubstituted or substituted with, *e.g.*, halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, acetylene, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Cycloalkyl" refers to a divalent cyclic or polycyclic alkyl group containing 3 to 15 carbon atoms.

"Substituted cycloalkyl" refers to a cycloalkyl group comprising one or more substituents with, *e.g.*, halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, acetylene, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Cycloheteroalkyl" refers to a cycloalkyl group wherein one or more of the ring carbon atoms is replaced with a heteroatom (*e.g.*, N, O, S or P).

"Substituted cycloheteroalkyl" refers to a cycloheteroalkyl group as herein defined which contains one or more substituents, such as halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Alkyl cycloalkyl" denotes the group -R-cycloalkyl where cycloalkyl is a cycloalkyl

group and R is a lower alkyl or substituted lower alkyl. Cycloalkyl groups can optionally be unsubstituted or substituted with *e.g.* halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Alkyl cycloheteroalkyl" denotes the group -R-cycloheteroalkyl where R is a lower alkyl or substituted lower alkyl. Cycloheteroalkyl groups can optionally be unsubstituted or substituted with *e.g.* halogen, lower alkyl, lower alkoxy, alkylthio, amino, amido, carboxyl, acetylene, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

If the final 2,6,9-trisubstituted purine compound of this invention contains a basic group, then an acid addition salt of the composition may be prepared. Acid addition salts of the compounds of this invention are prepared in a standard manner in a suitable solvent from the parent compound and an excess of acid, such as, but not limited to, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, maleic, succinic, or methanesulfonic. The hydrochloric salt form is especially useful.

If the final 2,6,9-trisubstituted purine compound contains an acidic group, then cationic salts of the composition may be prepared. Typically the acidic parent compound is treated with an excess of an alkaline reagent, such as, but not limited to, hydroxide, carbonate or alkoxide, containing the appropriate cation such as but not limited to, Na^+, K^+, Ca^{+2} and $NH4^+$. Certain of the compounds form inner salts or zwitterions which are also acceptable.

The compounds of this invention are useful in inhibiting cell proliferation in mammals including humans. The 2,6,9-trisubstituted purines are useful in for example in treating

autoimmune diseases, e.g. rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, etc., in treating cancer, cardiovascular disease such as restenosis, host vs graft disease, gout, polycystic kidney disease and other proliferative diseases whose pathogenesis involves abnormal cell proliferation.

The method of treatment comprises the administration parenterally, and orally, of an effective quantity of the chosen compound of this invention, preferably dispersed in a pharmaceutical carrier. Therapeutically useful amounts of the composition of this invention will generally range from about 0.01 to about 100 mg/kg, but will be readily determined by one skilled in the art depending upon the route of administration, and the age and condition of the patient. Therapeutically useful amounts of the composition of this invention may be administered from one to ten times daily or more for acute or chronic disease. No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The compounds of this invention are also useful as antiinflammatory and antifungal agents. As such, the compositions of this invention are useful for treating antiinflammatory and fungal infections in humans, animals, and fungal infections in plants.

Pharmaceutical compositions including the compounds of this invention, and/or derivatives thereof, may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. If used in liquid form the compositions of this invention are preferably incorporated into a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water and buffered sodium or ammonium acetate solution. Such liquid formulations are

suitable for parenteral administration, but may also be used for oral administration.

It may be desirable to add excipients such as polyvinylpyrrolidinone, gelatin, hydroxycellulose, acaia, polyethylene glycol, mannitol, sodium chloride, sodium citrate or any other excipient known to one of skill in the art to pharmaceutical compositions including compounds of this invention. Alternatively, the pharmaceutical compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include, but are not limited to syrup, peanut oil, olive oil, glycerin, saline, alcohols and water. Solid carriers include, but are not limited to, starch, lactose, calcium sulfate, dihydrate, teffa alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as, but not limited to, glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 gram per dosage unit.

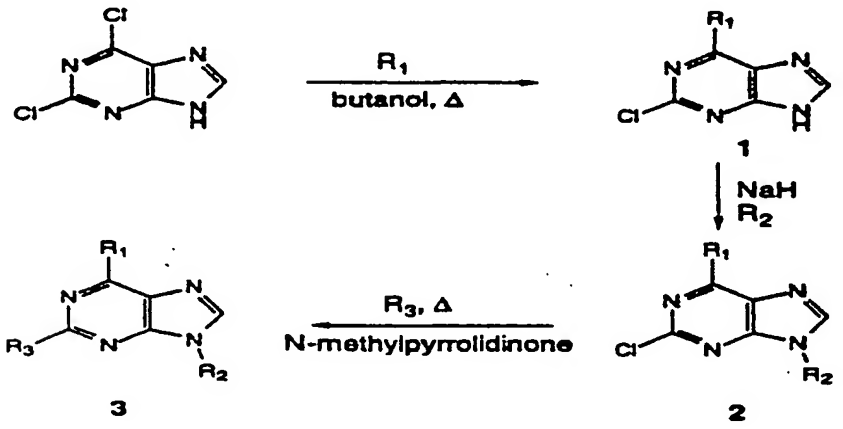
The pharmaceutical dosages are made using conventional techniques such as, but not limited to, milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly or filled into a soft gelatin capsule.

The Examples which follow serve to illustrate this invention. The Examples are intended to in no way limit the scope of this invention, but are provided to show how to make and use the compounds of this invention. In the Examples, all

temperatures are in degrees Centigrade. RT indicates room temperature.

EXAMPLE 1

The compounds of this invention are prepared by conventional methods of organic chemistry. The reaction sequence outlined in the synthesis scheme below is a general method useful for the synthesis of compounds of this invention. 2,6-dichloropurine is dissolved in butanol and the appropriate R_1 amine is added. After heating for several hours, the reaction mixture is cooled, and the compound 1 is obtained. To compound 1, is added, sodium hydride followed by R_2, and compound 2 is isolated. To compound 2, R_3 is added in solution with N-methylpyrrolidinone. The mixture is heated for an appropriate period followed by



purification leading to the desired compound.

The following compound was prepared according to the method above.

Preparation of 2-chloro-6-(4-methoxybenzylamino) purine (1).

The 2,6-dichloropurine (4.06 g, 21.5 mmol) was suspended in n-butanol (150 ml) and the 4-methoxybenzylamine was added (3.4 ml, 26 mmol). The solution turned clear and then cloudy a few minutes later. The solution was heated at 120°C for 2 hr and then cooled. The

n-butanol was evaporated followed by suspension of the residue in water and diethyl ether mixture. A solution of 2N NaOH (1.3ml, 26 mmol) was added and the solution stirred for 10 min before filtration. The filtered precipitate was washed with water and a small portion of ether and then dried under vacuum. The residual liquor was left overnight and more crystals were collected the next day and washed with diethyl ether. Yield = 71 %.

Preparation of 2-chloro-6-(4-methoxybenzylamino)-9-isopropylpurine (2)

2-chloro-6-(4-methoxybenzylamino) purine was suspended in dry DMF (5 ml) and treated with sodium hydride, 60% dispersion (82 mg, 2.06 mmol). The suspension was stirred for 30 min over which time it became a clear yellow/green solution. 2-Iodopropane (0.280 mL, 1.7 eq.) was added over 5 min and the resultant solution stirred for 2 days. Water was added and the solution and extracted with ethyl acetate. The organic layer was evaporated to give the product isopropyl purine (Yield = 508 mg, 89%).

Preparation of 2-diethanolamino-6-(4-methoxybenzylamino)-9-isopropylpurine, (3).

The purine (1.65g, 4.98 mmol) was dissolved in DMSO (12 mL) and diethanolamine (4 mL) and then heated at 140°C for 2-3 days and then at 160°C for 1 day. The solution was cooled and water saturated butanol was added (100 mL). The solution was then washed with water (3 x 50 mL), before being evaporated to give a brown oil. The residue was chromatographed over silica gel eluting with ethyl acetate, followed by 3% methanol in ethyl acetate to give the product (Yield = 730 mg, 37%) as a pale yellow oil. Yield =37%.

^{1}H-NMR(δ CDCl3): 7.29(br s 1H), 7.25(d, 2H), 6.94(br s. 1H), 6.83(d. 2H), 5.43(br s.<2H), 4.63(br s. 2H), 4.53(m 1H), 3.86(t. 4H), 3.76(m, 7H), 1.47(d 6H).

Table 1 identifies compounds of this invention that were prepared according to the

synthesis method set forth in this Example.

TABLE 1
Compounds Prepared By The Method of Example 1

R_1'-X	R2	R3
4-methoxybenzylamino	3-cyanopropyl	Cl
4-methoxybenzylamino	3-chloropropyl	Cl
4-methoxybenzylamino	benzyl	Cl
4-methoxybenzylamino	Methyl 4-carboxybenzyl	Cl
4-methoxybenzylamino	N-phthaloylethyl	Cl
4-methoxybenzylamino	isopropyl	Ethanolamine
4-methoxybenzylamino	isopropyl	Diethanolamine
4-methoxybenzylamino	3-methylbutyl	Cl
4-methoxybenzylamino	2-methylpropyl	Cl
4-methoxybenzylamino	cyclopentyl	Cl
4-methoxybenzylamino	3-nitrobenzyl	Cl
4-methoxybenzylamino	4-nitrobenzyl	Cl
4-methoxybenzylamino	ethyl	Cl
4-methoxybenzylamino	propyl	Cl
4-methoxybenzylamino	3-methylbenzyl	Cl
4-methoxybenzylamino	4-methylbenzyl	Cl
heptylamine	H	Cl
N-benzylhydroxylamine	H	Cl
propylamine	H	Cl
noradamantylamine	H	Cl
cyclobutylamine	H	Cl
3-methoxypropylamine	H	Cl
2-methoxyethylamine	H	Cl
cyclopentylamine	H	Cl

R_1'-X	R2	R3
2-amino-2-methyl-1-propanol	H	Cl
4-amino-1-benzylpiperidine	H	Cl
heptylamine	Me	Cl
N-benzylhydroxylamine	Me	Cl
propylamine	Me	Cl
noradamantylamine	Me	Cl
cyclobutylamine	Me	Cl
3-methoxypropylamine	Me	Cl
2-methoxyethylamine	Me	Cl
cyclopentylamine	Me	Cl
2-amino-2-methyl-1-propanol	Me	Cl
4-amino-1-benzylpiperidine	Me	Cl
2,4-dimethoxybenzylamine	Me	Cl
2-methoxybenzylamine	H	Cl
2-(aminomethyl)pyridine	H	Cl
3,4-dimethoxyphenethylamine	H	Cl
3-(aminomethyl)pyridine	H	Cl
4-(aminomethyl)pyridine	H	Cl
6-amino-1-hexanol	H	Cl
phenethylamine	H	Cl
2-aminobenzothiazole	H	Cl
2,4-dimethoxybenzylamine	H	Cl
2-methoxybenzylamine	Me	Cl
2-(aminomethyl)pyridine	Me	Cl
3,4-dimethoxyphenethylamine	Me	Cl

R_1'-X	R2	R3
4-methoxybenzylamino	Me	Cl
3-(aminomethyl) pyridine	isopropyl	Ethylenediamine
4-(aminomethyl)pyridine	H	Cl
6-amino-1-hexanol	H	Cl
phenethylamine	H	Cl
2-aminobenzothiazole	H	Cl
4-methoxybenzylamino	H	Cl
3-phenyl-1-propylamine	isopropyl	3-pyrroline
2-aminoindane	H	Cl
4-methoxyphenethylamine	H	Cl
4-nitrobenzylamine	H	Cl
2,6-difluorobenzylamine	H	Cl
3-phenyl-1-propylamine	H	Cl
2-aminoindane	Me	Cl
4-methoxyphenethylamine	Me	Cl
4-nitrobenzylamine	Me	Cl
2,6-difluorobenzylamine	Me	Cl
aminomethylcyclopropane	Me	Cl
piperonylamine	H	Cl
1-aminomethylbenzenesulfonamide	H	Cl
aminomethylcyclohexanol	H	Cl
2-aminomethylbenzimidazole	H	Cl
cyclohexanmethanamine	H	Cl
4-methoxybenzylamino	H	Cl

R_1'-X	R2	R3
4-methoxybenzylamino	isopropyl	Serinol
aminomethylcyclopropane	isopropyl	1,3-Diamino-2-hydroxypropane
piperonylamine	Me	Cl
1-aminomethylbenzenesulfonamide	Me	Cl
aminomethylcyclohexanol	Me	Cl
2-aminomethylbenzimidazole	Me	Cl
cyclohexanmethanamine	Me	Cl
3-(aminomethyl)pyridine	Me	Cl
4-(aminomethyl)pyridine	2-methylpropyl	Cl
6-amino-1-hexanol	cyclopentyl	Cl
phenethylamine	propyl	Cl
2-aminobenzothiazole	ethyl	Cl
3-phenyl-1-propylamine	isopropyl	Cl
2-aminoindane	2-methylpropyl	Cl
4-methoxyphenethylamine	cyclopentyl	Cl
4-nitrobenzylamine	propyl	Cl
2,6-difluorobenzylamine	ethyl	Cl
4-methoxybenzylamino	isopropyl	Cl
Phenpropylamino	isopropyl	4-hydroxypiperidine
2-aminoindane	H	Cl
2-(4-methoxyphenyl)ethylamino	H	Cl
4-nitrobenzylamino	H	Cl
2,6-difluorobenzylamino	H	Cl

R_1'-X	R2	R3
4-methoxybenzylamino	H	Cl
4-methoxybenzylamino	isopropyl	3-(Benzylamino)propionitrile
Phenpropylamino	isopropyl	(R/S)-Leucinol
2-aminoindane	isopropyl	Cl
2-(4-Methoxyphenyl)ethylamino	isopropyl	Cl
4-nitrobenzylamino	isopropyl	Cl
2,6-difluorobenzylamino	isopropyl	Cl
4-methoxybenzylamino	isopropyl	Cl
4-methoxybenzylamino	isopropyl	Piperidine
4-methoxybenzylamino	isopropyl	3-hydroxypiperidine
Phenpropylamino	isopropyl	L-Histidinol
2-aminoindane	isopropyl	diethanolamine
4-methoxybenzylamino	isopropyl	diethanolamine
4-methoxybenzylamino	isopropyl	(S)-(-)-2-pyrrolidinemethanol
4-methoxybenzylamino	isopropyl	Morpholine
4-methoxybenzylamino	benzyl	diethanolamine
4-methoxybenzylamino	3-methylbutyl	diethanolamine
4-methoxybenzylamino	2-methylpropyl	diethanolamine
4-methoxybenzylamino	cyclopentyl	diethanolamine
4-methoxybenzylamino	3-nitrobenzyl	diethanolamine
4-methoxybenzylamino	4-nitrobenzyl	diethanolamine
4-methoxybenzylamino	ethyl	diethanolamine
4-methoxybenzylamino	propyl	diethanolamine
4-methoxybenzylamino	3-methylbenzyl	diethanolamine
heptylamine	4-methylbenzyl	diethanolamine

R_1'-X	R2	R3
N-benzylhydroxylamine	Me	diethanolamine
propylamine	Me	diethanolamine
noradamantylamine	Me	diethanolamine
cyclobutylamine	Me	diethanolamine
3-methoxypropylamine	Me	diethanolamine
2-methoxyethylamine	Me	diethanolamine
cyclopentylamine	Me	diethanolamine
2-amino-2-methyl-1-propanol	Me	diethanolamine
4-amino-1-benzylpiperidine	Me	diethanolamine
4-methoxybenzylamino	Me	diethanolamine
4-methoxybenzylamino	isopropyl	2-pyrrolidinol
2,4-dimethoxybenzylamine	isopropyl	Tryptamine
2-methoxybenzylamine	Me	diethanolamine
2-(aminomethyl)pyridine	Me	diethanolamine
3,4-dimethoxyphenethylamine	Me	diethanolamine
3-(aminomethyl)pyridine	Me	diethanolamine
4-(aminomethyl)pyridine	Me	diethanolamine
6-amino-1-hexanol	Me	diethanolamine
phenethylamine	Me	diethanolamine
2-aminobenzothiazole	Me	diethanolamine
3-phenyl-1-propylamine	Me	diethanolamine
2-aminoindane	Me	diethanolamine
4-methoxyphenethylamine	Me	diethanolamine
4-nitrobenzylamine	Me	diethanolamine
2,6-difluorobenzylamine	Me	diethanolamine

R_1'-X	R2	R3
aminomethylcyclopropane	Me	diethanolamine
piperonylamine	Me	diethanolamine
1-aminomethylbenzenesulfonamide	Me	diethanolamine
aminomethylcyclohexanol	Me	diethanolamine
2-aminomethylbenzimidazole	Me	diethanolamine
cyclohexanmethanamine	Me	diethanolamine
3-(aminomethyl)pyridine	Me	diethanolamine
4-(aminomethyl)pyridine	2-methylpropyl	diethanolamine
6-amino-1-hexanol	cyclopentyl	diethanolamine
phenethylamine	propyl	diethanolamine
2-aminobenzothiazole	ethyl	diethanolamine
3-phenyl-1-propylamine	isopropyl	diethanolamine
2-aminoindane	2-methylpropyl	diethanolamine
4-methoxyphenethylamine	cyclopentyl	diethanolamine
4-nitrobenzylamine	propyl	diethanolamine
2,6-difluorobenzylamine	ethyl	diethanolamine
4-methoxybenzylamino	isopropyl	diethanolamine
4-methoxybenzylamino	isopropyl	1-amino-1-cyclopentanemethanol
4-methoxybenzylamino	isopropyl	(+-)-2-piperidinemethanol
cyclopropyl	isopropyl	(+-)-3-Amino-1,2-propanediol
piperonylamino	isopropyl	Cl
4-sulfaminobenzylamino	isopropyl	Cl
cyclohexanolmethylamino	isopropyl	Cl

R_1'-X	R2	R3
2-amino benzimidazolo	isopropyl	C1
cyclohexylmethylamino	isopropyl	C1
3-phenylpropylamino	isopropyl	C1
cyclopropylmethylamino	cyclopentyl	C1
piperonylamino	isopropyl	diethanolamine
4-methoxybenzylamino	isopropyl	diethanolamine
4-methoxybenzylamino	isopropyl	Disopropylamine
4-methoxybenzylamino	isopropyl	Trans-2-aminocyclohexanol
4-methoxybenzylamino	isopropyl	(R)-2-Amino-3-phenyl-1-propanol
4-methoxybenzylamino	isopropyl	(4S,5S)-(+)-5-amino-2,2-dimethyl-4
4-methoxybenzylamino	isopropyl	1-(3-aminopropyl)imidazole
4-methoxybenzylamino	isopropyl	4-hydroxy-4-phenylpiperidine
4-methoxybenzylamino	isopropyl	S-Benzyl-L-cysteinol
4-methoxybenzylamino	isopropyl	(+-)-Epinephrine
4-methoxybenzylamino	isopropyl	Diallylamine
4-methoxybenzylamino	isopropyl	Piperazine
4-methoxybenzylamino	isopropyl	(+-)-(Methylaminomethyl)benzylalcohol
4-methoxybenzylamino	isopropyl	(S)-(+)-2-(Anilinomethyl)pyrrolidine
4-methoxybenzylamino	isopropyl	4-(Allylamino)-4-methyl-2-pentanol
4-methoxybenzylamino	isopropyl	3-(2-hydroxyethylamine)propan-1-ol

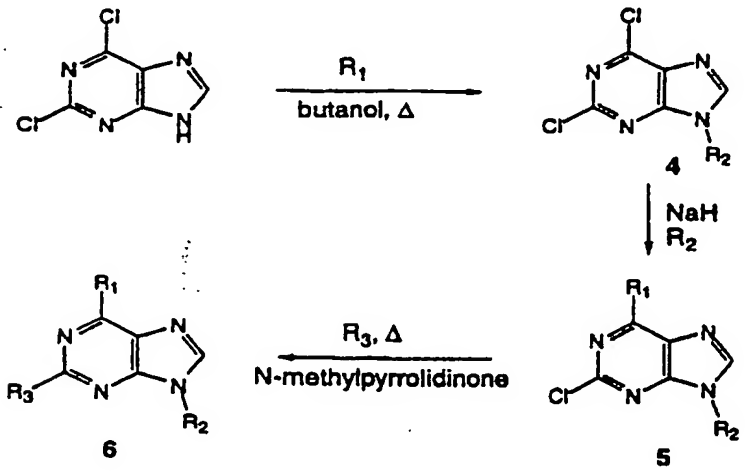
R_1'-X	R2	R3
4-methoxybenzylamino	isopropyl	1,1'-dimethyl-1,1'-dipropyl-2,2'-imidodiethanol
4-methoxybenzylamino	isopropyl	3,3'-iminodi-2-butanol
4-methoxybenzylamino	Me	ethanolamino
4-chlorobenzyloxy	H	Cl
4-chlorobenzyloxy	Me	Cl
4-chlorobenzylamino	Trifluoromethyl	Cl
4-methoxybenzylamino	Trifluoromethyl	Cl
4-methoxybenzylamino	benzyl	Cl
4-methoxybenzylamino	isopropyl	2-aminoethylamino
4-methoxybenzylamino	2-O-TBDMS-ethyl	diethanolamino
4-methoxybenzylamino	perfluoroisopropyl	Cl
4-methoxybenzylamino	perfluoroisopropyl	diethanolamino
4-methoxybenzylamino	2-hydroxyethyl	diethanolamino
4-methoxybenzylamino	isopropyl	1,3-diamino-2-hydroxpropane
4-methoxybenzylamino	isopropyl	N-(4-hydroxypiperidino)
4-methoxybenzylamino	isopropyl	N-pyrrolidino
3-phenylpropylamino	H	Cl
2-aminoindanyl	H	Cl
2-(4-methoxyphenyl) ethylamino	H	Cl
4-nitrobenzylamino	H	Cl
2,6-difluorobenzylamino	H	Cl
4-methoxybenzylamino	isopropyl	N-(2-cyanopropyl)-N-(3-pyridylmethyl)-amino
4-methoxybenzylamino	isopropyl	2-(hydroxymethyl)-3-methylbutan-2-amino
3-phenylpropylamino	isopropyl	Cl
2-aminoindanyl	isopropyl	Cl
2-(4-methoxyphenyl)ethylamino	isopropyl	Cl
4-nitrobenzylamino	isopropyl	Cl
2,6,difluorobenzylamino	isopropyl	Cl

R_1'-X	R2	R3
4-methoxybenzylamino	isopropyl	2-(5-imidazolemethyl) ethanolamino
3-phenylpropylamino	isopropyl	diethanolamino
4-methoxybenzylamino	isopropyl	N-(3-hydroxpyrrolidino)
4-methoxybenzylamino	isopropyl	2-(3-indole) ethylamino
4-methoxybenzylamino	isopropyl	2,3-dihydroxypropylamino
3-phenylpropylamino	cyclopentyl	Cl
4-methoxybenzylamino	isopropyl	N-benzyl-N-2-hydroxyethylamino
4-methoxybenzylamino	oleyl	Cl
4-methoxybenzylamino	2-naphthylmethyl	Cl
4-methoxybenzylamino	4-phenylbenzyl	Cl
4-methoxybenzylamino	1-naphthylmethyl	Cl
4-methoxybenzylamino	4-methylstilbene	Cl
4-methoxybenzylamino	epoxymethyl	Cl
4-methoxybenzylamino	2,3-dihydroxpropyl	diethanolamino
4-methoxybenzylamino	4-phenylbenzyl	diethanolamino
4-methoxybenzylamino	2-phenylbenzyl	diethanolamino
4-methoxybenzylamino	2-naphthylmethyl	diethanolamino
4-methoxybenzylamino	1-naphthylmethyl	diethanolamino
4-methoxybenzylamino	4-methylstilbene	diethanolamino
4-methoxybenzylamino	oleyl	diethanolamino
4-phenylbenzylamino	isopropyl	3-amino-1,2-propanediol
4-phenylbenzylamino	isopropyl	hexanolamino
4-phenylbenzylamino	isopropyl	bis(methoxyethyl) amino
4-phenylbenzylamino	isopropyl	furfurylamino
4-phenylbenzylamino	isopropyl	diethylamino
4-phenylbenzylamino	isopropyl	ethanolamino
4-phenylbenzylamino	isopropyl	morpholino
4-phenylbenzylamino	isopropyl	2,4-dimethoxybenzylamino
4-phenylbenzylamino	isopropyl	4-trifluoromethoxybenzylamino
4-phenylbenzylamino	isopropyl	diisopropanolamino
4-phenylbenzylamino	isopropyl	2-amino-1,3-propanediol
4-phenylbenzylamino	isopropyl	diallyl amino
4-bromobenzylamino	isopropyl	Cl

R_1'-X	R2	R3
4-bromoanilino	isopropyl	Cl
4-bromobenzylamino	isopropyl	diethanolamino
4-bromoanilino	isopropyl	diethanolamino
N-methyl-4-phenylbenzylamino	isopropyl	Cl
4-phenylanilino	isopropyl	diispropanolamino
N-methyl-4-phenylbenzylamino	isopropyl	diethanolamino
benzylamino	ethyl	ethanolamino
4-methylbenzylamino	methyl	ethanolamino
4-ethylbenzylamino	methyl	ethanolamino
4-bromanlino	isopropyl	4-bromoanilino

EXAMPLE 2

This Example describes a method for preparing compounds of this invention. The synthesis method disclosed in this Example is only slightly modified from that disclosed in Example 1.



The following compound was prepared according to the method above.

Preparation of 2,6-dichloro-9-isopropylpurine (4).

To a solution of 0.67g of 2,6-dichloropurine in 5mL of dry DMF at room temperature was added 0. 16gms (l. 1 eq.) of 50% sodium hydride/oil powder. Upon cessation of hydrogen evolution, a large excess (2 mL) of isopropyl iodide was added to the anionic solution. This reaction solution was stirred for three days at ambient temperature. The reaction was quenched with 30 mL of water and extracted with ethyl acetate (3X50 mL). The organic extracts were combined and back washed with 3X50 mL of water followed by 20 mL of brine. The ethyl acetate solution was dried over anhydrous magnesium sulfate and evaporated. The compound was subjected to variable gradient flash

chromatography on silica gel with hexane/ethyl acetate mixtures and yielded 0.37gms of desired N-9 product (45%) and 0.08gms of the N-7 isomer(10%).

Preparation of 2-chloro-6-anilino-9-isopropylpurine (5).

2,6-dichloro-9-isopropylpurine (0.019 g, 0.081 mmol) was dissolved in butanol (0.5 ml) and aniline (0.044 ml, 0.244 mmol) was added. The reaction mixture was heated to 120°C for 10 hr, cooled, diluted with EtOAc and washed 3 times with water. The mixture was dried over $MgSO_4$ and concentrated to an off white solid.

Preparation of 2-diethanolamino-6-(4-phenylanilino)-9-isopropylpurine (6).

A solution of 67mgs of 2,6-dichloro-N-9-isopropylpurine and 100mgs of 4-phenylaniline in 1 mL of n-octanol was heated to 80°C for 24 hours. The n-octanol was removed in vacuo and then replaced with 1 mL of 40% diethanolamine in DMSO. The solution was heated at 130°C for 48 hours. The reaction was cooled to ambient temperature then diluted with 10 mL of water and subsequently extracted with ethyl acetate (3X30 mL). The organic extracts were combined and back washed with 3X20 mL of water followed by 10 mL of brine. The ethyl acetate solution was dried over anhydrous magnesium sulfate and filtered and the solvent was evaporated. The 65mgs of crude product was crystallized from THF-ether solution to yield 28mgs of pure product(23%).

Table 2 below identifies compounds of this invention that were prepared according to the general synthesis method set forth in this Example.

TABLE 2
Compounds Prepared By The Method Of Example 2

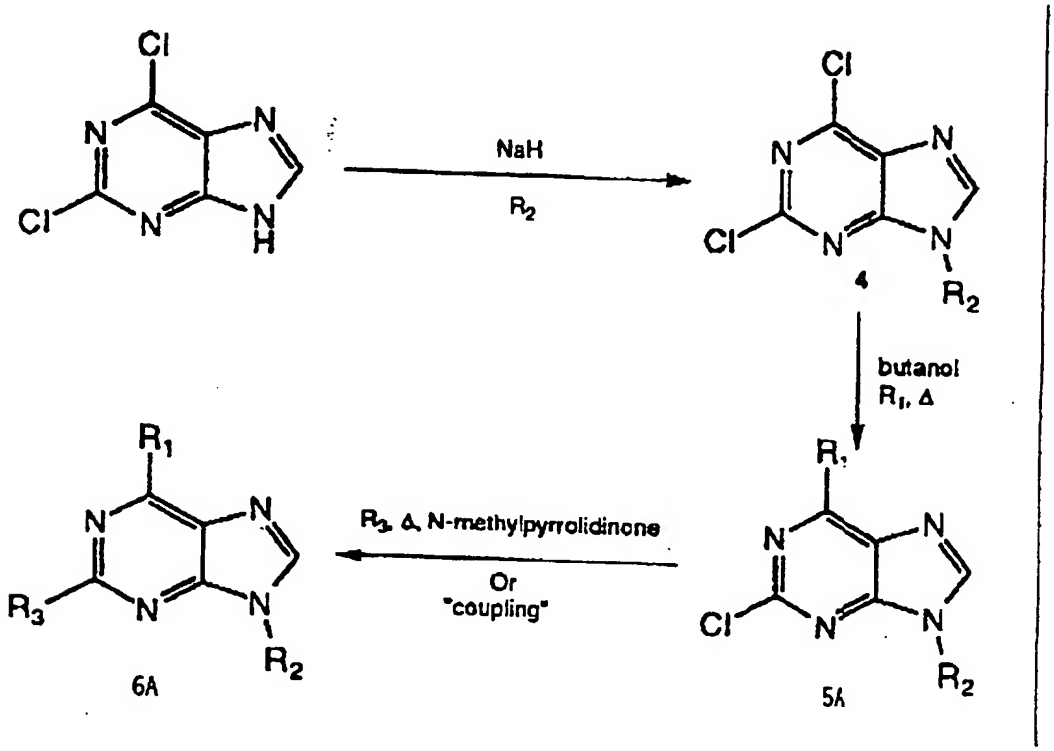
R_1'-X	R2	R3
8-aminoquinoline	isopropyl	Cl
6-aminoquinoline	isopropyl	Cl

R_1'-X	R2	R3
3-aminoquinoline	isopropyl	Cl
anilino	isopropyl	Cl
3,5-dinitroaniline	isopropyl	Cl
4-butylaniline	isopropyl	Cl
8-aminoquinoline	isopropyl	diethanolamine
6-aminoquinoline	isopropyl	diethanolamine
3-aminoquinoline	isopropyl	diethanolamine
aniline	isopropyl	diethanolamine
3,5-dinitroaniline	isopropyl	diethanolamine
4-butylaniline	isopropyl	diethanolamine
2-amino-6-ethoxybenzothiazole	isopropyl	Cl
4-(2-amniomethyl)morpholine	isopropyl	Cl
4-(1-aminomethyl)benzenesulfonamide	isopropyl	Cl
4-bromoaniline	isopropyl	diethanolamine
3,4-dichloroaniline	isopropyl	diethanolamine
2-(2-aminoethyl)-1-methylpyrrolidine	isopropyl	diethanolamine
3-bromoaniline	isopropyl	Cl
4-anisidine	isopropyl	diethanolamine
4-iodoaniline	isopropyl	Cl
3-iodoaniline	isopropyl	Cl
m-anisidine	isopropyl	Cl
1-(2-aminoethyl)piperidine	isopropyl	diethanolamine
1-(2-aminoethyl)pyrrolidine	isopropyl	diethanolamine
1-aminoindane	isopropyl	diethanolamine
2-amino-6-ethoxybenzothiazole	isopropyl	diethanolamine
4-(2-amnioethyl)morpholine	isopropyl	diethanolamine
4-(1-aminomethyl)benzenesulfonamide	isopropyl	diethanolamine
4-bromoaniline	isopropyl	diethanolamine
3,4-dichloroaniline	isopropyl	diethanolamine
2-(2-aminoethyl)-1-methylpyrrolidine	isopropyl	diethanolamine

R_1'-X	R2	R3
3-bromoaniline	isopropyl	diethanolamine
4-anisidine	isopropyl	diethanolamine
4-iodoaniline	isopropyl	diethanolamine
3-iodoaniline	isopropyl	diethanolamine
m-anisidine	isopropyl	diethanolamine
1-(2-aminoethyl)piperidine	isopropyl	diethanolamine
1-(2-aminoethyl)pyrrolidine	isopropyl	diethanolamine
1-aminoindane	isopropyl	diethanolamine
3-iodoaniline	isopropyl	diethanolamine
3-iodoaniline	isopropyl	diethanolamine
3-phenoxyaniline	isopropyl	diethanolamine
4-iodoaniline	isopropyl	diethanolamine
4-phenoxyaniline	isopropyl	diethanolamine
3-phenoxyaniline	isopropyl	diethanolamine
4-iodoanline	isopropyl	diethanolamine
2-fluorenylamino	isopropyl	diethanolamine
1-fluorenylamino	isopropyl	diethanolamine
2-anthracenylamino	isopropyl	diethanolamine
1-anthracenylamino	isopropyl	diethanolamine
2-(6-ethoxybenzothiazole)amino	isopropyl	diethanolamine
2-phenylbenzylamino	isopropyl	diethanolamine
4-phenylbenzylamino	isopropyl	diethanolamine
2-naphthylmethylamino	isopropyl	diethanolamine
1-naphthylmethylamino	isopropyl	diethanolamine

EXAMPLE 3

This Example describes a method for preparing compounds of this invention. The synthesis method disclosed in this Example is only slightly modified from that disclosed in Example 1.



The following compound was prepared according to the method above.

Preparation of 2,6-dichloro-9-isopropylpurine (4).

The 2,6-dichloropurine (5.00 g, 26.46 mmol) was suspended in 55 ml of dry DMF at room temperature and treated with sodium hydride, 60% dispersion (1.27 g, 31.75 mmol) added in portions. After stirring for 1 hr, 2-iodopropane (4.5 ml, 44.98 mmol) was added and

the reaction stirred for 2 days. The reaction was poured into diethyl ether and washed once with saturated sodium bicarbonate solution and once with water. The mixture was dried over anhydrous sodium sulfate and concentrated in vacuo. The concentrate was chromatographed over silica gel eluting with 10% acetone in dichloromethane solution to give the desired N-9 alkylation product as a white solid. Yield = 47%.

Preparation of 2-chloro-6-(4-methylmercapto) anilino-9-isoproplypurine (5A).

2,6-Dichloro-9-isopropylpurine (0.15 g, 0.649 mmol) was dissolved in n-butanol (4 ml) and 4-(methylmercapato) aniline (0.089 ml, 0.714 mmol) and triethylamine (0.20 ml, 1.43 mmol) were added. The reaction mixture was heated at 80° overnight. The cooled reaction was diluted ethyl acetate and washed 1 x 1M HCl, 1 x saturated sodium bicarbonate, and 1 x brine before being dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed over silica gel and eluting with 2% methanol in dichloromethane to give the desired product as a white solid. Yield = 83%.

Preparation of 2-diethanolamine-6-(4-methylmercapto) anilino-9-isopropylpurine (6A).

The purine (0.18 g, 539 mmol) was dissolved in N-methylyrrolidinone (3 ml) and diethanolamine (1 ml) and then heated at 120°C overnight. The cooled reaction was poured into diethyl ether and washed three times with water before drying over anhydrous sodium sulfate and concentrating in vacuo. The residue was chromatographed over silica gel eluting with 5% methanol in dichloromethane to give the desired product as an off-white solid. Yield = 82 %. ^{1}H-NMR(δ, $CDCl_3$) : 8.08(s,1H), 7.58(d, 2H), 7.47(s,1H), 7.18(d, 2H), 4.95(br s, <2H), 4.52(m, 1H), 3.94(m, 4H), 3.83(m,4H), 2.43(s, 3H), 1.47(d, 6H).

Preparation of 4-(2-thienyl) benzonitrile.

Some R_1' groups must first be synthesized before reacting with the 2,6-dichloro-9-isopropylpurine. These groups can be synthesized through various coupling methods and other synthetic procedures known to those skilled in the art of organic synthesis.

To a pressure tube was added 4-bromobenzonitrile (0.20 g, 1.10 mmol), tetrakis(triphenylphosphine) palladium (0) (0.127 g, 0.1 eq) and 2-thiopheneboronic acid (0.211 g, 1.65 mmol). The reaction was flushed under vacuum and flushed with dry nitrogen three times. Following flushes, ethyleneglycol dimethyl ether (5.5 ml) and an aqueous solution of sodium carbonate (2.53 ml, 1M) were added to the tube. The tube was then sealed and heated at 80°C overnight. The cooled reaction was the diluted with diethyl ether and washed twice with water before drying over sodium sulfate and concentrating in vacuo. The residue was chromatographed over silica gel eluting with 10% ethyl acetate in hexane to give the desired product as a white solid. Yield = 84%.

Preparation of 4-(2-thienyl) benzylamine.

The 4-(2-thienyl)benzonitrile (0.086 g, 0.464 mmol) was dissolved in dry tetrahydrofuran (1.6 ml) before lithium aluminum hydride (0.46 ml, 0.464 mmol, 1 M in THF) was added dropwise. The reaction was allowed to stir at room temperature overnight. TLC (5% methanol in dichloromethane) still showed starting material remaining. Another 1 eq of LAH was added. After an additional hour, the reaction was quenched by the Fieser and Fieser method using wager (17.46µl), aqueous sodium hydroxide solution (17.46µl, 15% soln.), and water (52.37 µl) added sequentially to the reaction. The reaction was then diluted with diethyl ether and water and extracted twice with diethyl ether before drying over sodium

sulfate and concentrating in vacuo. The residue was carried on crude without any further purification. Yield = 89%.

Table 3 below identified compounds of this invention that were prepared according to the general synthesis method set forth in this Example.

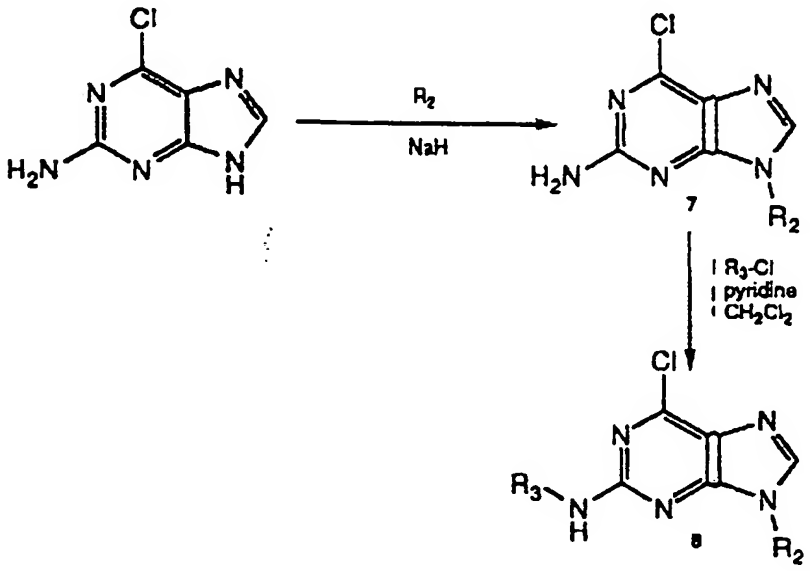
Table 3
Compounds Prepared By The Method of Example 3

R_1'-X	R2	R3
Cl	Me	Cl
ethanolamino	Me	ethanolamino
cyclopropylmethylamino	isopropyl	Cl
cyclopropylmethylamino	isopropyl	diethanolamino
3,5-dinitroanilino	isopropyl	Cl
3-phenoxyanilino	isopropyl	Cl
4-iodoanilino	isopropyl	Cl
3-aminoquinolino	isopropyl	Cl
3,5-dinitroanilino	isopropyl	diethanolamino
Cl	epoxymethyl	Cl
4-methoxybenzylamino	2,3-dihydroxypropyl	diethanolamino
4-phenylanilino	isopropyl	diethanolamino
4-phenylbenzylamino	isopropyl	Cl
2-naphthalenylmethylamino	isopropyl	Cl
1-naphthalenylmethylamino	isopropyl	Cl
2-phenylbenzylamino	isopropyl	Cl
3-quinolinylamino	isopropyl	diethanolamino
5-quinolinylamino	isopropyl	diethanolamino
6-quinolinylamino	isopropyl	diethanolamino
8-quinolinylamino	isopropyl	diethanolamino
n-butylamino	isopropyl	Cl
4-(2-thiophenyl)benzylamino	isopropyl	deithanolamino
4-(2-thiophenyl)benzylamino	isopropyl	Cl
3-thiomethoxyanilino	isopropyl	Cl
4-thiomethoxyanilino	isopropyl	Cl
3-thiomethorxyanilino	isopropyl	diethanoamino
4-thiomethoxyanilino	isopropyl	diethanoamino
4-(2-pyridinyl) benzylamino	isopropyl	Cl
3-methoxybenzylamino	isopropyl	Cl
3,4-dimethoxybenzylamino	isopropyl	Cl
3,4,5-trimethoxyenzylamino	isopropyl	Cl
3-methoxybenzylamino	isopropyl	diethanolamino

R_1'-X	R2	R3
3,4-dimethoxybenzylamino	isopropyl	diethanolamino
3,4,5-trimethoxbenzylamino	isopropyl	diethanolamino
4-(3-thiophenyl)benzylamino	isopropyl	Cl
4-(4-methoxphenyl) benzylamino	isopropyl	Cl
4-(4-bromophenyl) benzylamino	isopropyl	diethanolamino
4-(3- methoxyphenyl) benzylamino	isopropyl	diethanolamino
4-(4-methoxypheny) benzylamino	isopropyl	diethanolamino
4-(3-thiophenyl) benzylamino	isopropyl	diethanolamino
4-(3-methylpheny) benzylamino	isopropyl	Cl
4-(4-methylphenyl) benzylamino	isopropyl	Cl
4-(4-trifluoromethylphenyl) benzylamino	isopropyl	Cl
3-(4 nitrilophenyl)anilino	isopropyl	Cl
3-(4-nitrilophenyl)anilino	isopropyl	diethanolamino
4-(2-pyridinyl)benzylamino	isopropyl	Cl
4-(2-pryidinyl)benzylamino	isopropyl	diethanolamino

EXAMPLE 4

This Example describes a method for preparing compounds of this invention. The synthesis method disclosed in this Example is only slightly modified from that disclosed in Example 1.



The following compound was prepared according to the method above.

Preparation of 2-amino-6-chloro-9-methylpurine (7).

The 2-amino-6-chloropurine (1.08 g, 6.4 mmol) was suspended in dry DMF (75 ml) and treated with sodium hydride, 60% dispersion (0.28 g, 7 mmol). The suspension was stirred for 15 min before iodomethane (0.44 ml, 7.06 mmol) was added and the resulting yellow solution stirred for 1 hr 45 min. The solid was filtered and the filtrate evaporated before addition of water for 10 min. The resulting solid was filtered and dried overnight to

give the product as a mixture of N-7 and N-9 alkylation products. The residual liquor was left overnight and more crystals were collected the next day and dried. Yield = 77%.

Preparation of 6-chloro-2-(2-methoxyacetylamino)-9-methylpurine (8).

The mixture of isomers from above was dissolved in dichloromethane and pyridine (2 eq) followed by treatment with methoxyacetyl chloride (4 eq). The reaction was stirred at room temperature until complete. The reaction was evaporated and filtered through a plug of silicia gel eluting with 2% methanol in dichloromethane followed by purification on a chomatotron using silica gel and eluting with 2% methanol in dichloromethane to isolate the desired product. Yield = 31%.

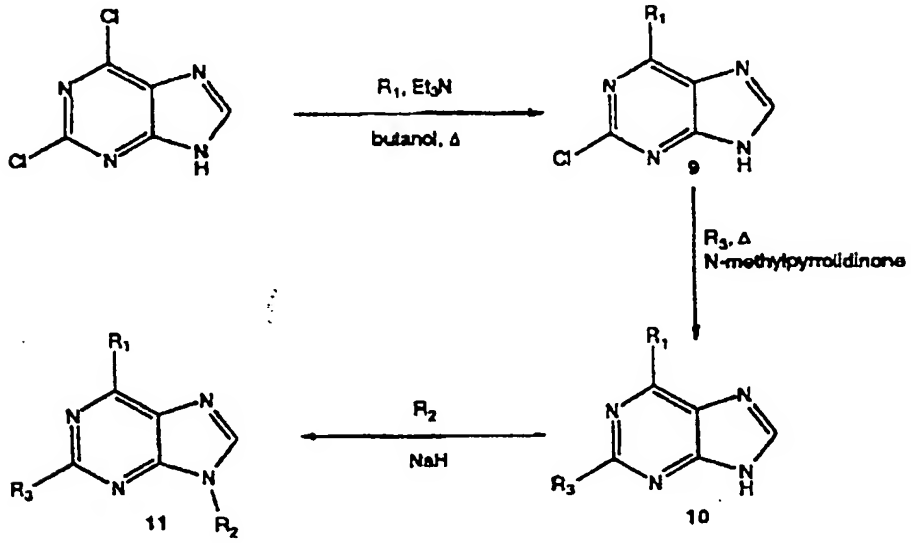
Table 4 identifies compounds of this invention that were prepared according to the synthesis method set forth in this Example.

Table 4
Compounds Prepared By The Method of Example 4

R1	R2	R3
Cl	Me	H
Cl	Me	2-methoxyacetylamino

EXAMPLE 5

This Example describes a method for preparing compounds of this invention. The synthesis method disclosed in this Example is only slightly modified from that disclosed in Example 1.



The following compound was prepared according to the method above.

Preparation of 2-chloro-6-(4-phenyl benzylamino) purine (9).

The 2,6-dichloropurine (5.0 g, 26.45 mmol) was suspended in n-butanol (50 ml) and the 4-phenylbenzylamine (6.61 g, 29.1 mmol) and triethylamine (4.1 ml, 29.1 mmol) were added. The solution was heated at 120°C overnight then cooled. Filtered off product using excess n-butanol and washed precipitate with 100 ml 1M HCl and 200 ml water. The solid was dried in vaccum over overnight at 70°C to give the desired product as a pale yellow solid. Yield = 99%.

Preparation of 2-diethanolamino-6-(4-phenyl benzylamino) purine (10).

The 2-chloro-6-(4-phenyl benzylamino) purine (2.0 g, 5.96 mmol) was added together with diethanolamine (11.4 ml, 119.2 mmol) and N-methylpyrrolidinone (10 ml) and heated at 120°C overnight. The cooled reaction was poured into dichloromethane and washed twice with water. The organic layer was dried with anhydrous sodium sulfate and concentrated in vacuo to give the desired product as a pale green solid which was further dried in vacuum oven at 70°C for 2 days.

Preparation of 2-diethanolamino-6-(4-phenyl benzylamino)-9-methylpurine (11).

The 2-diethanolamino-6-(4-phenyl benzylamino) purine (0.050 g, 0.124 mmol) was dissolved in dry DMF and treated wit sodium hydride, 60% dispersion (5.5 mgs, 0.136 mmol) for 1 hr. iodomethane (0.009 ml, 0.148 mmol) was added and the resultant solution stirred at room temperature overnight. Poured reaction into diethyl ether and washed twice with saturated sodium bicarbonate solution before drying over anhydrous sodium sulfate and concentrating in vacuo. The residue was chromatographed over silica gel eluting with 5% methanol in dichloromethane to give the produce as a white solid. Yield = 63%.

^{1}H-NMR(δ, CDCl3): 7.55 (m,4H), 7.41 (m, 4H) 7.35(m, 4H), 6.41 (br s, < 1H), 5.10(br s, <2H), 4.72 (br s, 2H), 3.86 (m, 4H), 3.74(m, 4H), 3.59(s, 3H).

Table 5 identified compounds of this invention that were prepared according to the synthesis method set forth in this Example.

Table 5
Compounds Prepared By The Method of Example 5

R_1'-X	R2	R3
4-phenylbenzylamino	methyl	diethanolamino
4-phenylbenzylamino	cyclopentyl	diethanolamino
4-phenylbenzylamino	allyl	diethanolamino
4-phenylbenzylamino	benzyl	diethanolamino
4-phenylbenzylamino	3-methylbutyl	diethanolamino

4-phenylbenzylamino	isobutyl	diethanolamino
4-phenylbenzylamino	t-butylacetate	diethanolamino
4-phenylbenzylamino	methylacetate	diethanolamino
4-phenylbenzylamino	cyclobutyl	diethanolamino
4-phenylbenzylamino	ethyl	diethanolamino
4-phenylbenzylamino	propyl	diethanolamino

EXAMPLE 6

Composition of this invention were evaluated in the following assays.

CDK2 assays:

Compositions of this invention were assayed to determine their CDK2 inhibitory activity. The assay system (total volume of 50 µl) contained 50 mM Tris-Cl, pH 7.4, 10 mM $MgCl_2$, 5 mM DTT, 1 µg of histone H1, 30 µM ATP (1 µCi of gamma^{32}P labeled ATP), 10 µg of BSA and 1 ng of purified CDK2. After incubation at 30°C for 30 min, the reaction was terminated by the addition of 10 µl of 10% TCA and the samples were blotted onto to nitrocellulose filters. These filters were washed extensively in 10% TCA and assayed for radioactivity. Blanks contained no enzyme. To ascertain the potency of various compounds of this invention, the compounds were added to the above assay at concentrations ranging from 100 to 0.02 µg/ml. After incubation at 30 min., the assay tubes were processed as above. In all assays, various concentrations of olomoucine were added and were used as a standard positive control. The IC_{50} (enzyme) listed in Table 6 is defined as the concentration required to inhibit CDK2 activity by 50%.

EXAMPLE 7

Cell Proliferation Assays:

Early passage rat aortic smooth muscle cells (CV Therapeutics Cell repository) were seeded in 48 well dishes (Falcon, ml/well) at a density of 20,000 cells/ml of DME containing 5% heat inactivated bovine serum. The cells were incubated in a standard tissue culture incubator for 48 hr. The medium was aspirated and the wells were replenished with 0.2 ml of fresh medium. Compounds of this invention were added at concentrations ranging from 100 to 0.37 µg/ml. After 48 hr incubation, the medium was aspirated and the cultures were treated with 0.2 ml of saline 0.25 µl of phenozine methosulfate solution containing MTS (Cell Titer 96® Aqueous Non-radioactive cell proliferation assay kit, Catalog # G 5430, Promega, 2800 Woods Hollow Road, Madison, WI 53711-5399). The IC_{50} cells listed in Table 6 is defined as the concentration required to inhibit cell proliferation by 50%. Olomoucine at various concentrations was added and was used as a standard positive control.

TABLE 6
Bioactivity of Selected Representatives of this Invention

R_1'-X	R2	R3	IC_{50} (µg/mL) enzyme	IC_{50} (µg/mL) cells
benzylamino	Me	ethanolamino	7	70
4-methoxybenzylamino	H	Cl	60	NA
4-methoxybenzylamino	Me	Cl	6	>70
4-methoxybenzylamino	Me	ethanolamino	4	48
4-chlorobenzyloxy	H	Cl	60	NA
4-chlorobenzyloxy	Me	Cl	60	NA
4-chlorobenzyloxy	trifluoromethyl	Cl	>60	NA
4-methoxybenzylamino	isopropyl	Cl	4	77

R,'-X	R2	R3	IC_{50} (μg/mL) enzyme	IC_{50} (μg/mL) cells
4-methoxybenzylamino	isopropyl	ethanolamino	4	43
4-methoxybenzylamino	Me	diethanolamino	4	48
4-methoxybenzylamino	2-methylpropyl	Cl	60	>70
ethanolamino	Me	ethanolamino	>60	>70
4-methoxybenzylamino	trifluoromethyl	Cl	>60	>70
4-methoxybenzylamino	benzyl	Cl	>60	>70
ethanolamino	H	benzylamino	>60	NA
4-methoxybenzylamino	isopropyl	diethanolamino	0.2	2.1
4-methoxybenzylamino	perfluoroisopropyl	Cl	>45	NA
4-methoxybenzylamino	perfluoroisopropyl	diethanolamino	40	NA
4-methoxybenzylamino	ispropyl	3-pyrroline	1	12.5
4-methoxybenzylamino	hydroxyethyl	diethanolamino	0.5	62
4-methoxybenzylamino	isopropyl	serinol	0.4	15
4-methoxybenzylamino	isopropyl	1,3-diamino-2-hydroxypropane	0.6	25
4-methoxybenzylamino	3-cyanopropyl	Cl	>60	NA
4-methoxybenzylamino	3-chloropropyl	Cl	>60	NA
4-methoxybenzylamino	benzyl	Cl	>60	NA
4-methoxybenzylamino	Methyl 4-carboxybenzyl	Cl	>60	NA
4-methoxybenzylamino	Naphthaloylethyl	Cl	>60	NA
4-chlorobenzylamino	Trifluoromethyl	Cl	1	NA
4-methoxybenzylamino	isopropyl	N-(2-cyanopropyl)-N-(3-pyridylmethyl)-amino	1	NA
4-methoxybenzylamino	isopropyl	2-(hydroxymethyl)-3-methylbutan-2-amino	1	NA
4-methoxybenzylamino	isopropyl	3-hydroxypiperidino	1	NA
cyclohexylmethylamino	isopropyl	Cl	1	NA
piperonylamino	isopropyl	diethanolamino	0.8	NA
4-methoxybenzylamino	isopropyl	diisopropanolamino	0.8	NA
anilino	isopropyl	Cl	1	NA

R_1'-X	R2	R3	IC_{50} (μg/mL) enzyme	IC_{50} (μg/mL) cells
4-methoxybenzylamino	isopropyl	N-benzyl-N-2-hydroxyethylamino	1	NA
4-phenylanilino	isopropyl	diethanolamino	0.6	NA
4-phenylbenzylamino	isopropyl	diethanolamino	0.6	NA
4-phenylbenzylamino	isopropyl	3-amino-1,2-propanediol	0.6	NA
4-(2-thiophenyl)benzylamino	isopropyl	diethannolamino	0.5	NA
4-(4-methylphenyl) benzylamino	isopropyl	diethanolamino	0.6	NA
4-(4-trifluoromethylphenyl) benzyalmino	isopropyl	diethanolamino	0.6	NA
4-thiomethoxyanilino	isopropyl	Cl	0.6	NA
3-(4-nitrilophenyl) anilino	isopropyl	diethanolamino	0.5	NA
3-thiomethoxyanilino	isopropyl	diethanolamino	0.1	NA
4-thiomethoxyanilino	isopropyl	diethanolamino	0.07	NA
3-methoxybenzylamino	isopropyl	Cl	0.9	NA
4-(2-pyridinyl) benzylamino	isopropyl	diethanolamino	0.16	NA
3-methoxybenzylamino	isopropyl	diethanolamino	0.5	NA

The inhibition of cell proliferation properties of the compounds of this invention are demonstrated by their ability to inhibit cell proliferation in the range of about 0.05 μg/ml to 100 μg/ml, preferably less than 0.5 μg/ml.

EXAMPLE 7

A compound of this invention was evaluated for effectiveness using the Murine Leukemia Model. The Murine Leukemia Model is a standard model used in the evaluation of antitumor agents. CDF1 mice were injected ip with L1210 cells ($1x10^3$ cells/mouse). Twenty-four hours later, these mice were treated with various doses (ip) of compound 3 of Example 1 in saline. The dosing regimen used in this study is outlined in Table 7, below. Mice were dosed with compound 3 daily or on alternate days. Control mice received saline. After 7 days, dosing was suspended and survival monitored.

Table 7

Treatment		N	Median survival time Days	T/Cx100
Saline control		7	10 (9-13)	100
Compound 3	0.5 mg/kg bid	7	11 (10-15)	110
	1.0 mg/kg bid	7	13 (11-13)	130
	2 mg/kg bid	7	12 (10-14)	120
	4 mg/kg - days 1,3,5,7	7	13 (10-15)	130
	8 mg/kg - days 1,3,5,7	7	13 (12-16)	130

The results indicate that rats administered compound 3 survived longer than the control rats.

EXAMPLE 8

This example measured the effect of an acute local delivery of compound 3 of Example 1 in reducing neointima formation following balloon angioplasty in the rat carotid artery model. In this example, the left common carotid arteries of adult male rats (n=10 per experimental group) were surgically injured using a Fogarty arterial embolectomy catheter. Immediately after injury, the common carotid artery was bisected with a vascular clamp, thereby establishing an untreated and treated segment. A drug delivery catheter was then inserted into the distal half of the common carotid. After drug delivery, the catheter was removed and excess drug was washed out by removing the vascular clamp and re-establishing blood flow before closing the artery. The animals were allowed to recover for 14 days before harvesting the common carotid artery. The harvested tissue was sectioned and the neointimal area was digitized and measured with a computer planimetery system. For each animal, 15 measurements were averaged for the untreated segment and 15 for the treated.

The results of this Example are found in Figure 1. According to Figure 1, administering compound 3 of Example 1 to a damaged carotid artery reduced the neointimal area about 88% in comparison to the 6% reduction produced by the saline vehicle alone.

EXAMPLE 9

IκB-α Kinase Assays:

Compositions of this invention were assayed to determine their IκB-α kinase inhibitory activity. The human umbilical vein endothelial cell line (HUVEC) used in these studies was purchased from Clonetics (San Diego, CA) and was maintained in endothelial cell growth medium supplemented with 2% fetal bovine serum, 10ng/ml human recombinant epidermal growth factor, 1 μg/ml hydrocortisone, 50 μg/ml gentamicin, 50 ng/ml amphotericin B and 12 μg/ml bovine brain extract at 37°C in a tissue culture incubator. All growth media and supplements were purchased from Clonetics (San Diego, CA). *E. coli* lipopolysaccharide (LPS) serotype 0111:B4 was purchased from Sigma (Saint Louis, MI). All other chemicals were of reagent grade.

Preparation of cell Lysate: Monolayers (75 cm^2) of HUVEC cells were treated with LPS (100 ng/ml) for 5 minutes after which the cell media was rapidly removed and the monolayer washed three times with ice cold PBS. The cell layer was scraped into 10 ml PBS and the cells pelleted by centrifugation (3000 rpm, 5 min, 4°C). Cell lysate was prepared by incubating the cell pellet in 0.2 ml lysis buffer (20mM HEPES, pH7.3, 50mM NaCl, 10mM $MgCl_2$, 1mM EDTA, 1mM EGTA, 1mM sodium orthovanadate, 10mM β-glycerophospate, 1mM phenylmethylsulfonylfuoride, 1mM dithiothreitol, 0.5% Nonidet P-40 for 15 minutes at 37°C for frequent vortexing. Cell debris was removed from the sample by microcentrifugation (10,000xg, 15 minutes, 4°C) and the supernatant was "precleared" by the addition of 100 ml of a suspension of sepharose 4B in lysis buffer and mixing gently for 1 hour at 4°C. The speharose 4B beads were removed by microcentrifugation and the supernatant aliquotted and stored at 80°C.

Solid Phase IκB-α kinase assay: 1 μg of GST- IκB-α, corresponding to full length IκB-α of human origin, (Santa Cruz Biotechnology,) was incubated with 20 μl of a 50% slurry of glutathione S sepharose 4B (Pharmacia) in reaction buffer (20mM HEPES, pH7.3, 10mM $MgCl_2$, 15mM β-glycerophosphate, 0.5mM sodium orthovanadate, 0.5mM EGTA) for 30 minutes at room temperature. The GST- IκB-bead complex was the washed three times with 0.5 ml of reaction buffer by resuspension and microcentrifugation. 10μg of HUVEC cell lysate protein in 100μl of reaction buffer was then added to the GST- IκB-bead complex and the mixture incubated with gentle mixing at 4°C for 1 hour. The bead complex was then washed three times with reaction buffer containing 0.2 M NaCl and once with reaction buffer alone. Finally the bead complex was resuspended in 20μl of reaction buffer containing 5μCi [y-^{32}P]ATP (>5000 ci/mmol, New England Nuclear Corp. Boston, MA) and incubated at room temperature for 15 minutes. The reaction was terminated by the addition of 10μl of SDS-PAGE sample buffer and boiled for 3 minutes before separation by SDS-PAGE (10-20% gradient Readygel, BioRad). Following electrophoresis the gel was fixed (50% methanol 10% acetic acid) for 15 minutes, washed three times for 5 minutes each with distilled H_2O and treated with 5% glycerol for 15 minutes before drying down and exposing to film for autoradiography (X-OMAT XAR-5 Kodak).

In gel kinase assay: IκB-α isozymes were assayed for activity using a modification of previously published methods (11, 19, 20). Briefly duplicate samples of the IκB-glutathione sepharose 4B bead complex were prepared as described above and were separated by electrophoresis through a 12% SDS-PAGE gel which had been polymerised in the presence of 15 μg/ml GST- IκB-α. Following electrophoresis the gel was washed gently twice for 30 minutes each with 50mM Tris-HCl pH8.0, 5mM β-mercaptoethanol; 20% isopropanol to

remove SDS. Proteins were then denatured within the gel by incubation for 45 minutes in 100ml 50mM Tris-HCl pH8.0; 5mM β-mercaptoethanol; 0.04% Tween 40. The gel was then cut in half to separate the duplicate samples, one half was incubated in 10 ml reaction buffer alone and the other in 10 ml reaction buffer containing 10μg/ml of 2-diethanolamino-6(4-phenyl anilino)-9-isopropyl purine (compound 6 of Example 2) for 1 hour at room temperature which 10μCi[y-^{32} P]ATP was added and the incubations continued for a further hour at room temperature. The gels were then subjected to multiple 15 minute washes of 100ml each 5% trichloroacetic acid containing 1% sodium pyrophosphate until 1 ml of wash solution gave close to background radioactivity. The gels were then dried down and exposed to file for autoradiograhy.

Preparation of 2-diethanolamino-6-(4-phenybenzylamino)-9-isopropyl purine Epoxy activated Sepharose 6B Affinity Matrix. Freeze dried epoxy activated Sepharose 6B (Pharmacia LKB, Piscataway, NJ) was chosen for the coupling reaction due to its ability to form an ether bond between an hydroxyl-containing ligand and the epoxide group on the sepharose. The gel was swollen according to the manufacturer's instructions, (100mg) of compound 6 of Example 2 was dissolved in 1ml coupling solution (1.2:1 v/v dimethylformamide : 0.1N NaOH) and mixed with 0.5ml of swollen gel at pH 10-11 for 72 hours at room temperature with gentle agitation. Excess reactive groups were blocked with 1M ethanolamine for 4 hours at 50°C and the gel slurry was poured into 1 ml syringe column. The resin was activated with three alternating cycles of twenty column volumes each of pH 4.0 (0.1M acetate, 0.5M NaCl) and pH 8.0 (0.1M Tris-HCl, 0.5M NaCl) buffers followed by twenty column volumes of reaction buffer (20mM HEPES, pH7.3, 10mM $MgCl_2$, 15mM β-glycerophophate, 0.5mM sodium orthovanadate, 0.5mM EGTA). The column was stored at 4°C in reaction buffer containing

0.5% sodium azide and regenerated prior to each use with alternating cycles of low and high pH as described above.

Activated HUVEC cell lysate (500μg protein in 1ml reaction buffer) was passed over the CVT-1545 sepharose matrix sequentially five times and the flow through was saved (unbound material). The matrix was then washed three times with 1ml of reaction buffer (wash 1-3) then three times each with reaction buffer containing 0.5M NaCl (eluate 1-3). Aliquots (20μl from 1ml) of each sample were assayed for their ability to phosphorylate at GST- IκB-sepharose bead complex and analyzed by SDS-PAGE as described above.

Assay of affinity enriched IκB-α kinase. The bulked 0.5 M NaCl eluates from the affinity matrix were used as the source of enzyme for development of an IκB-α kinase filter assay. Each reaction contained affinity enriched IκB-α kinase (1μg protein), 10ng GST IκB-α kinase and 0.5μCi[y-^{32}P]ATP (>5000 Ci/mmol, New England Nuclear Corp, Boston, MA) in 20μl reaction buffer. The reaction was incubated for 15 minutes at room temperature and was terminated by the addition of 2μl 0.5M EDTA. Reaction mixtures were blotted onto phosphocellulose disks (Gibco BRL Life Technologies, Gaithersburg, MD) and the filters washed three times with 0.15M phosphoric acid with gentle shaking for 15 minutes (up to ten filters were washed with 300 ml of 0.15M phosphoric acid.) Following a third wash the filters were air dried, added to scintillation fluid and assayed by liquid scintillation spectrometry.

Electrophoretic Mobility Shift Assay: Nuclear extracts were prepared using a high-salt buffer extraction procedure. 10 pmol of double stranded NF-κB consenses oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3'))Promega) was 5' end labeled with 5μCi [y-^{32}P]ATP (>5000 Ci/mmol, New England Nuclear Corp, Boston, MA) by incubaton with T4 polynucleotide kinase for 1 hr at 37°C. Unincorporated nucleotides were removed by pasing

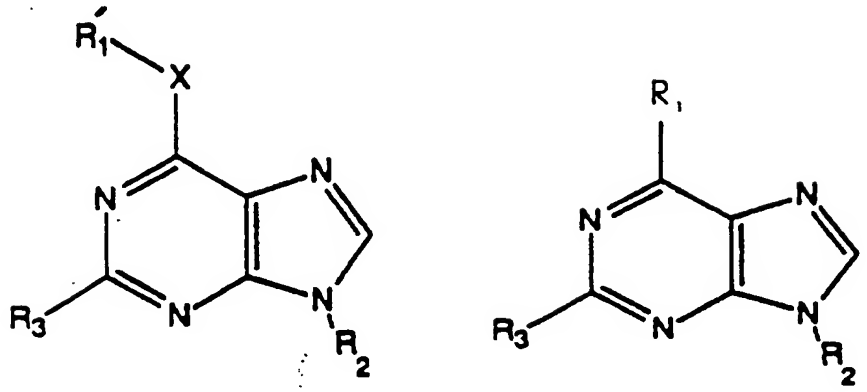
the reaction mixture over 1ml Sephadex G-5-spin column. Binding assays were performed at room temperature for 1 hr and consisted of 10μg nuclear extract protein, 1μg salmon sperm DNA, and $5x10^4$ cpm of ^{32}P labeled consensus of oligonucleotide in the presence and absence of fifty fold unlabeled oligonucleotide. DNA-protein complexes were resolved by 8% non denaturing polyacrylamide gel electrophoresis, the gels were dried onto filter paper and visualized by autoradiography.

Table 8
Enzyme Activity of Selected Representatives of this Invention

R_1'-X	R2	R3	IC50(μM) enzyme
4-phenylbenzylamino	isopropyl	diethanolamino	1.1
4-phenylbenzylamino	isopropyl	diethylamino	>2.4
4-phenylbenzylamino	isopropyl	ethanolamino	2.5
4-bromoanilino	isopropyl	diethanolamino	14
4-(3-methoxphenyl) benzylamino	isopropyl	diethanolamino	>10
4-(4-methoxphenyl) benzylamino	isopropyl	diethanolamino	11
3-(4-nitrilophenyl) anilino	isopropyl	diethanolamino	2.2
4-thiomethoxyanilino	isopropyl	diethanolamino	12.4
4-(2-pyridinyl) benzylamino	isopropyl	diethanolamino	4.5

What we claim is:

1. A 2,6,9-trisubstituted purine composition of matter having the following formula:



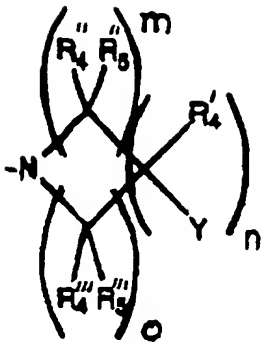
(I)

R_1 is halogen or R'_1-X wherein X is a amino, oxo, thio, or sulfone moiety.

R'_1 is a lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, substituted cycloheteroalkyl, aryl, substituted aryl, heterocycle, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, heteroalkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, or alkyl cycloheteroalkyl, each having from 1 to 20 carbon atoms;

R_2 is hydrogen, or hydrocarbon compound selected from the group lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl, aryl, substituted aryl, heterocycle, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, heteroalkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, or alkyl cycloheteroalkyl wherein each hydrocarbon compound has from 1 to 20 carbon atoms;

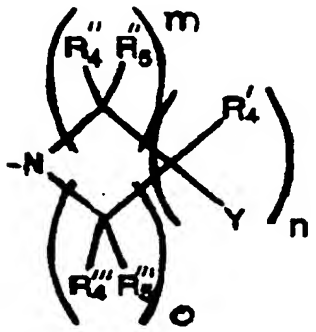
R_3 is halogen, hydroxyl, thio, alkoxy, alkylthio, lower alkyl, $-NR_4R_5$ or a component having the formula:



where m=1-3, n=1-3, o=1-3, Y=carbonyl, -NR_4R_5, hydroxyl, thiol, alkoxy, alkythio, and wherein R_4 and R_5 are each independently hydrogen, or a hydrocarbon selected from the group including lower alkyl, substituted lower alkyl, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, or cyano, wherein each hydrocarbon has from 1 to 20 carbon atoms wherein when Y is carbonyl, R'_4 does not exist in the composition, R_4'' and R_5'' may be a single oxygen atom, R_4''' and R_5''' may be a single oxygen atom, and wherein when R_3 is 2-hydroxyethylamino and R_2 is methyl, R_1'-X is not amino, 3-methyl-2-butenylamino, benzylamino, or m-hydroxybenzyl-amino, when R_3 is 2-hydroxyethylamino, when R_2 is isopropyl, R_1'-X is not benzylamino, m-hydroxybenzylamino, or 3-methylbutylamino, when R_3 is 2-hydroxyethylamino and R_2 is 2-hydroxyethyl, R_1'-X is not benzylamino and when R_3 is selected from the group consisting of 2-propanol-2-methylamino and 2-dimethylaminoethylamino and R_2 is methyl, then R_1'-X is not benzylamino.

2. The 2,6,9-trisubstituted purine composition of claim 1 wherein X is amino.

3. The 2,6,9-trisubstituted purine composition of claim 1 wherein R_3 is a component having the formula:



where m=1-3, n=1-3, o=1-3, Y=carbonyl, $-NR_4R_5$, hydroxyl, thiol, alkoxy, alkythio, and wherein R_4 and R_5 are each selected from the group including hydrogen, lower alkyl, substituted lower alkyl, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, or cyano wherein when Y is carbonyl, R'_4 does not exist in the composition, R_4'' and R_5'' may be a single oxygen atom, R_4''' and R_5''' may be a single oxygen atom.

4. The 2,6,9-trisubstituted purine composition of claim 3 wherein R_1' is selected from the group consisting of aralkyl and heteroarylalkyl.

5. The 2,6,9-trisubstituted purine composition of claim 4 wherein R_1' is selected from the group consisting of aralkyl, unsubstituted pyridylalkyl and substituted pyridylalkyl and wherein R_2 is selected from the group consisting of lower alkyl, substituted lower alkyl,

and alkyl cycloalkyl.

6. The 2,6,9-trisubstituted purine composition of claim 3 wherein R_1' is selected from the group consisting of aryl, heterocyclc, heteroaryl, substituted heteroaryl, and substituted aryl.

7. The 2,6,9-trisubstituted purine composition of claim 3 wherein R_1' is selected from the group consisting of aryl, unsubstituted pyridyl, substituted pyridyl, and substituted aryl, and R_2 is selected from the group consisting of lower alkyl, substituted lower alkyl, and alkyl cycloalkyl.

8. The 2,6,9-trisubstituted purine composition of claim 2 wherein R_3 is $-NR_4R_5$ wherein R_4 and R_5 are each selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, alkoxy, amino, amido, carboxyl, cycloalkyl, substituted cycloalkyl, heterocycle, cycloheteroalkyl, substituted cycloheteroalkyl, acyl, aryl, substituted aryl, aryloxy, hetaryl, substituted hetaryl, aralkyl, heteroaralkyl, alkyl alkenyl, alkyl alkynyl, alkyl cycloalkyl, alkyl cycloheteroalkyl, or cyano.

9. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is selected from the group consisting of aralkyl, substituted pyridylalkyl, and unsubstituted pyridylalkyl, R_2 is selected from the group consisting of lower alkyl, substituted lower alkyl, cycloalkyl, and substituted cycloalkyl, R_4 is a substituted lower alkyl having from 2 to 6 carbon atoms, and R_5 is selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, cycloalkyl, aryl cycloalkyl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl, heteroalkyl, heteroaralkyl, and substituted cycloalkyl.

10. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is selected from the group consisting of aryl, substituted aryl, pyridyl, and substituted pyridyl, R_2 is

selected from the group consisting of lower alkyl, substituted lower alkyl, cycloalkyl, alkyl cycloalkyl, and substituted cycloalkyl, R_4 is a substituted lower alkyl having from 2 to 6 carbon atoms, and R_5 is selected from the group consisting of hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, cycloalkyl, aryl cycloalkyl, heterocycle, substituted heterocycle, heteroaryl, substituted heteroaryl, heteroalkyl, heteroaralkyl, and substituted cycloalkyl.

11. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is selected from the group consisting of aralkyl, pyridylalkyl, and substituted pyridylalkyl, R_2 is selected from the group consisting of lower alkyl, substituted lower alkyl, and alkyl cycloalkyl, and R_4 and R_5 are each a substituted lower alkyl having from 2 to 6 carbon atoms.

12. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is CH_2 - Aryl or CH_2 - substituted aryl, R_2 is lower alkyl or substituted lower alkyl, and R_4 and R_5 are each $-CH_2\ CH_2OH$, $-CHR'CH_2OH$, or $-CH_2\ CHR'OH$ wherein R' is hydrogen or alkyl having from 1 to 6 carbon atoms.

13. The 2,6,9-trisubstituted purine composition of claim 12 wherein R_2 is isopropyl.

14. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is selected from the group consisting of aryl, substituted aryl, pyridyl, and substituted pyridyl, R_2 is selected from the group consisting of lower alkyl, substituted lower alkyl, and alkyl cycloalkyl, and R_4 and R_5 are each a substituted lower alkyl having from 2 to 6 carbon atoms.

15. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is aryl or substituted aryl, R_2 is lower alkyl, or substituted lower alkyl, and R_4 and R_5 are each CH_2CH_2OH , $-HR'CH_2OH$, or $-CH_2CHR'OH$ wherein R' is hydrogen or alkyl having from 1

to 6 carbon atoms.

16. The 2,6,9-trisubstituted purine composition of claim 15 wherein R_2 is isopropyl.

17. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is benzyl substituted with a halogen, alkoxy, phenyl, pyridyl or nitro group, R_2 is isopropyl, and R_4 and R_5 are each $-CH_2CH_2OH$.

18. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is phenyl substituted with a halogen, alkoxy, phenyl, pryidyl or nitro group, R_2 is isopropyl, and R_4 and R_5 are each $-CH_2CH_2OH$.

19. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is biphenyl, R_2 is isopropyl, and R_4 and R_5 are each $-CH_2CH_2OH$.

20. The 2,6,9-trisubstituted purine composition of claim 8 wherein R_1' is selected from the group consisting of 3-thiomethoxyphenyl, 4-thiomethoxyphenyl 4-bromophenyl, 4-phenylbenzyl, 4-methoxybenzyl, 4-biphenyl, 3-methoxybenzyl, 4-(2-thienyl)benzyl, 4-(4-methyl)phenylbenzyl, 4-(4-trifluoromethyl)phenylbenzyl, 4-(4-nitrilo)phenylbenzyl, 4-(2-pyridinyl)benzyl, piperonyl, 3-methoxbenzyl, 4-chlorobenzyl, and 4-nitrobenzyl, R_2 is isopropyl, and R_4 and R_5 are both CH_2CH_2OH.

21. The 2,6,9-trisubstituted purine composition of claim 20 wherein R_1' is 4-methoxybenzyl.

22. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-phenylbenzyl.

23. The 2,6,9-trisubstituted purine composition of claim 20 wherein R_1 is 4-methoxybenzyl.

24. The 2,6,9-trisubstituted-purine composition of claim 20 wherein R'$_1$ is 4-biphenyl.

25. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 3-methoxybenzyl.

26. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-(2-thienyl)benzyl.

27. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-(4-methyl)phenylbenzyl.

28. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-(4-trifluoromethyl)phenylbenzyl.

29. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-(4-nitrilo)phenylbenzyl.

30. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-(2-pyridinyl)benzyl.

31. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is piperonyl.

32. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 3-thiomethoxphenyl.

33. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-thiomethoxyphenyl.

34. The 2,6,9-trisubstituted purine composition of claim 20 wherein R'$_1$ is 4-bromophenyl.

35. A cationic salt of the composition of claim 1.

36. An acid addition salt of the composition of claim 1.

37. A method for inhibiting cell proliferation in mammals comprising administering a therapeutically effective amount of the composition of claim 1 to the mammal.

38. The method of claim 37 wherein the therapeutically effective amount ranges from about 0.001 to about 100 mg/kg weight of the mammal.

39. The method of claim 37 wherein the composition is administered to a mammal suffering from a cell proliferation disorder selected from the group consisting of rheumatoid arthritis, lupus, type I diabetes, multiple sclerosis, cancer, restenosis, host graft disease, and gout.

40. The method of claim 39 wherein the cell proliferation disorder is restenosis.

41. The method of claim 39 wherein the cell proliferation is disorder cancer.

42. The method of claim 39 wherein the cell proliferation disorder is polycystic kidney disease.

43. The method of claim 39 wherein the mammal is a human.

44. A pharmaceutical composition of matter comprising the composition of claim 1 and one or more pharmaceutical excipients.

45. The pharmaceutical composition of matter of claim 43 wherein the pharmaceutical composition is in the form of a solution.

46. The pharmaceutical composition of matter of claim 43 wherein the pharmaceutical composition is in the form of a tablet.

47. An antifungal agent useful for treating fungal infections in humans, and animals comprising the composition of claim 1.

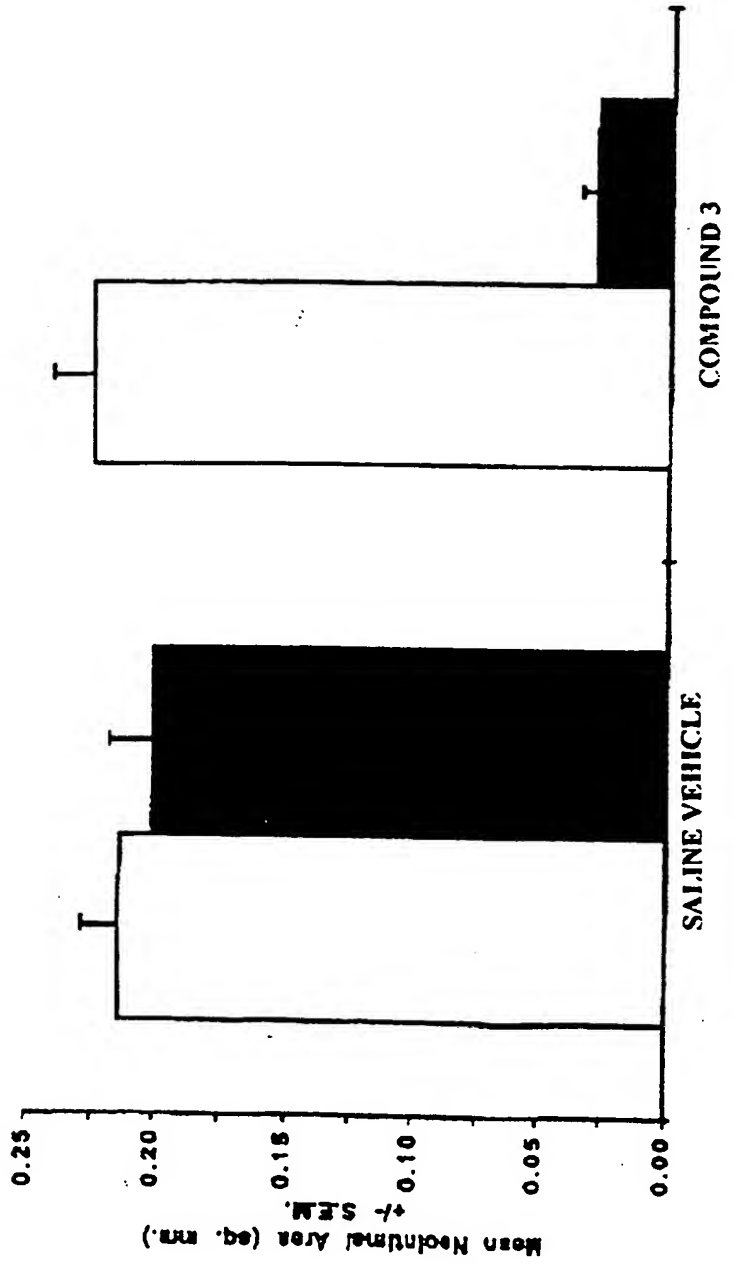

Mean Nocturnal Area (sq. mm.)
+/- S.E.M.
0.25
0.20
0.15
0.10
0.05
0.00
SALINE VEHICLE
COMPOUND 3

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13386

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :544/264, 265, 276, 277; 514/261, 262, 263, 265, 266

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/261, 262, 263, 265, 266
544/264, 265, 276, 277

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	VESELY et al. Inhibition of cyclin-dependent Kinases by Purine Analogues. Eur. J. Biochem. 1994. Volume 224, pages 771-786. See species 51.	1, 2, 8, 9, 35-39, 41, 43, 47 ---------- 3-7, 10-34, 40, 42, 44-46
X -- A	US 5,508,277 A (REGNIER et al.) 16 April 1996. See Examples 1, 2 and other purine examples.	1-4, 35-39, 41, 43-47 ---------- 5-34, 40, 42

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 SEPTEMBER 1997	31 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARK L. BERCH aco Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13386

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- A	US 5,498,819 A (KANEKO et al.) 12 March 1996. See column 15, compound 31-33; column 16, #101; #144, and column 2, line 5.	1, 2, 8, 35-39, 41, 43-47 --- 3-7, 9-34, 40, 42
X -- A	BRESHEARS et al. The Aminolysis Of Certain Chlorosubstituted Purines, Journal of the American Chemical Society. 20 July 1959. Volume 81, pages 3789-3792. See Indicated Table 2 species.	1-4, 8 --- 5-7, 9-47
X -- A	US 4,028,358 A (LIOTTA) 07 June 1977. See column 3 and column 5 starting materials.	1-34 --- 35-47
X -- A	US 4,405,781 A (BADER et al.) 20 September 1983. See column 2, line 38.	1-36 --- 37-47
X -- A	WO 93/17020 A1 (THE WELLCOME FOUNDATION LILITED) 02 September 1993. See Formula I; page 4, first full paragraph; page 5, species a), b), c) and d).	1, 2, 8, 35, 44-47 --- 3-7, 9-34, 37-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13386

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/52; C07D 473/40, 473/38, 473/36, 473/34, 473/32, 473/30, 473/24, 473/22, 473/20, 473/18, 473/16, 473/04